This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS

1. ***(currently** amended)* A method of determining the relative copy number (CN) of a first nucleotide sequence I (NucSeqI) in a sample using an amplification technique, said method comprising the steps of:

(1) adding to the sample nucleotides, primers, polymerase, a probe directed to NucSeqI and NucSeqI', comprising a first fluorophore and a quencher, and optionally, any additional reagents required for amplification, wherein the sample comprises a chromosome-derived second nucleotide sequence II (NucSeqII) and a probe directed to NucSeqII and NucSeqII' comprising a second fluorophore and a quencher, wherein the first fluorophore and the second fluorophore are different;

(2) ~~performing one or more amplification cycles to amplify the NucSeqI,~~ carrying out the following amplification steps in one or more amplification cycles:

(a) amplifying NucSeqI,

(b) amplifying NucSeqII,

(c) amplifying a third nucleotide sequence I' (NucSeqI'), corresponding to NucSeqI and present in a control sample, at multiple dilutions, wherein the relationship of NucSeqI and NucSeqI' is defined as

(A) NucSeqI hybridizes to the complement of NucSeqI', and

(B) NucSeqI' hybridizes to the complement of NucSeqI,

both under stringent hybridization conditions, and if NucSeqI and NucSeqI' differ in length, the shorter of the two is at most 30% shorter than the other; and

(d) amplifying a fourth nucleotide sequence II' (NucSeqII'), corresponding to NucSeqII and present in a control sample, at multiple dilutions, wherein the relationship of NucSeqII and NucSeqII' is defined as

(A) NucSeqII hybridizes to the complement of NucSeqII', and

(B) NucSeqII' hybridizes to the complement of NucSeqII,

both under stringent hybridization conditions, and if NucSeqII and NucSeqII' differ in length, the shorter of the two is, at most, 30% shorter than the other;

wherein

(i) NucSeqI' and NucSeqII' are both localized on a single vector in which the ratio of ~~concentration of~~ NucSeqI' to ~~the concentration of~~ NucSeqII' is known,

(ii) standard curves SC_I and SC_{II} comprising at least two reference points are generated by amplification of NucSeqI' and NucSeqII', respectively, at multiple dilutions, and

(iii) at least one pair of amplification reactions (a) and (b) or (c) and (d) is performed in a single container and monitored by fluorescence during amplification;[[, and]]

~~(iv) NucSeqI' and NucSeqII' are localized on a single vector; and~~

(3) determining [[from]] the results of the amplifications of step (2) expressed as threshold cycle (Ct);

(4) obtaining from the results in step (3) the following values:

~~concentrations of NucSeqI and NucSeqII using the respective standard curves SC_I and SC_{II}, to obtain the relative CN of NucSeqI with respect to NucSeqII by the formula:~~

$$\cancel{\text{Relative CN} = \frac{\text{Conc-I}_{SCI}}{\text{Conc-II}_{SCII}}}$$

~~wherein, in said formula,~~

~~(i) "relative CN" is the ratio of the CN of NucSeqI relative to the CN of NucSeqII in the sample;~~

(i[[i]]) "Conc-I_{SCI}" which is the concentration or quantity in the sample of NucSeqI determined from standard curve SC_I; and

(ii[[i]]) "Conc-II_{SCII}" which is the concentration or quantity in the sample of NucSeqII determined from standard curve SC_{II},
which standard curves express threshold cycle as a function of said concentration or quantity; and

(5) determining from the values obtained in step (4) the relative CN of NucSeqI with respect to NucSeqII by the formula:

$$\underline{\text{Relative CN}} = \frac{\underline{\text{Conc-I}_{SCI}}}{\underline{\text{Conc-II}_{SCII}}}$$

thereby determining the relative CN.

2. *(previously presented)* A method for determining the absolute CN of a nucleotide sequence NucSeqI in a sample, comprising:

(a) determining the relative CN using the method of claim 18, and

(b) multiplying the relative CN by the absolute CN of NucSeqII per cell.

3. ***(currently** amended)* A method according to claim 1, wherein at least two different NucSeqI' sequences, used for measuring a corresponding number of different NucSeqI sequences, are localized on a single vector.

4. *(previously presented)* A method according to claim 1 wherein the sequences of NucSeqI and NucSeqI' are the same.

5. *(previously presented)* A method according to claim 1 wherein the sequences of NucSeqII and NucSeqII' are the same.

6. ***(currently** amended)* A method according to claim 2, wherein at least two different NucSeqI' sequences, used for measuring a corresponding number of different NucSeqI, are localized on a single vector.

7. *(previously presented)* A method according to claim 2 wherein the sequences of NucSeqI and the NucSeqI' are the same.

8. *(previously presented)* A method according to claim 3 wherein the sequences of NucSeqI and the NucSeqI' are the same.

9. *(previously presented)* A method according to claim 6 wherein the sequences of NucSeqI and the NucSeqI' are the same.

10. *(previously presented)* A method according to claim 2 wherein the sequences of NucSeqII and the NucSeqII' are the same.

11. *(previously presented)* A method according to claim 3 wherein the sequences of NucSeqII and the NucSeqII' are the same.

12. *(previously presented)* A method according to claim 4 wherein the sequences of NucSeqII and the NucSeqII' are the same.

13. *(previously presented)* A method according to claim 6 wherein the sequences of NucSeqII and the NucSeqII' are the same.

14. *(previously presented)* A method according to claim 7 wherein the sequences of NucSeqII and the NucSeqII' are the same.

15. *(previously presented)* A method according to claim 8 wherein the sequences of NucSeqII and the NucSeqII' are the same.

16. *(previously presented)* A method according to claim 9 wherein the sequences of NucSeqII and the NucSeqII' are the same.

17. *(previously presented)* A method according to claim 1, wherein the sample is derived from cells.

18. *(previously presented)* A method according to claim 17, wherein an absolute CN of NucSeqII per cell is known.

19. *(previously presented)* A method according to claim 18, wherein at least two different NucSeqI' sequences used for measuring a corresponding number of different NucSeqI are localized on a single vector.

20. *(previously presented)* A method according to claim 18, wherein the sequences of NucSeqI and the NucSeqI' are the same.

21. *(previously presented)* A method according to claim 18 wherein the sequences of NucSeqII and the NucSeqII' are the same.

22. *(previously presented)* A method according to claim 19 wherein the sequences of NucSeqII and the NucSeqII' are the same.

23. *(previously presented)* A method according to claim 20 wherein the sequences of NucSeqII and the NucSeqII' are the same.

24. ***(currently** amended)* A method of determining the relative CN of a first nucleotide sequence I (NucSeqI) in a sample using an amplification technique, said method comprising the steps of:

(1) adding to the sample nucleotides, primers, polymerase, a probe directed to NucSeqI and NucSeqI' comprising a fluorophore and a quencher, and optionally, any additional reagents required for amplification, wherein the sample comprises a second nucleotide sequence II (NucSeqII) and a probe directed to NucSeqII and NucSeqII' comprising a second fluorophore and a quencher, wherein the first fluorophore and the second fluorophore are different;

(2) ~~performing one or more amplification cycles to amplify the NucSeqI,~~ carrying out the following amplification steps <u>in one or more amplification cycles</u>:

(a) amplifying NucSeqI,

(b) amplifying NucSeqII,

(c) amplifying a third nucleotide sequence I' (NucSeqI'), corresponding to NucSeqI and present in a control sample, at multiple dilutions, wherein the relationship of NucSeqI and NucSeqI' is defined as

(A) NucSeqI hybridizes to the complement of NucSeqI', and

(B) NucSeqI' hybridizes to the complement of NucSeqI,

both under stringent hybridization conditions, and if NucSeqI and NucSeqI' differ in length, the shorter of the two is<u>,</u> at most<u>,</u> 30% shorter than the other;

(d) amplifying a fourth nucleotide sequence II' (NucSeqII'), corresponding to NucSeqII and present in a control sample, at multiple dilutions, wherein the relationship of NucSeqII and NucSeqII' is defined as

(A) NucSeqII hybridizes to the complement of NucSeqII', and

(B) NucSeqII' hybridizes to the complement of NucSeqII,

both under stringent hybridization conditions, and if NucSeqII and NucSeqII' differ in length, the shorter of the two is at most 30% shorter than the other;

wherein

(i) NucSeqI' and NucSeqII' are both localized on a single vector in which the ratio of ~~concentration of~~ NucSeqI' to ~~the concentration of~~ NucSeqII' is known,

(ii) standard curves SC_I and SC_{II} comprising at least two reference points are generated by amplification of NucSeqI' and NucSeqII', respectively, at multiple dilutions,

(iii) at least one pair of amplification reactions (a) and (b) or (c) and (d) is performed in a single container and monitored by fluorescence during amplification, and

~~(iv) NucSeqI' and NucSeqII' are localized on a single vector; and~~

(3) determining [[from]] the results of the amplifications of step (2) expressed as threshold cycle (Ct);

(4) obtaining from the results in step (3) the following values:

~~concentrations of NucSeqI and NucSeqII using the respective standard curves SC_I and SC_{II}, to obtain the relative CN of NucSeqI with respect to NucSeqII by the formula:~~

$$\cancel{\text{Relative CN} = \frac{\text{Conc-I}_{SCI}}{\text{Conc-II}_{SCII}}}$$

~~wherein, in said formula,~~

~~(i) relative CN" is the ratio of the CN of NucSeqI relative to the CN of NucSeqII in the sample;~~

(i[[i]]) "Conc-I_{SCI}" which is the concentration or quantity in the sample of NucSeqI determined from standard curve SC_I; and

(ii[[i]]) "Conc-II_{SCII}" which is the concentration or quantity in the sample of NucSeqII determined from standard curve SC_{II}; and

(5) determining from the values obtained in step (4) the relative CN of NucSeqI with respect to NucSeqII by the formula:

$$\text{Relative CN} = \frac{\text{Conc-I}_{SCI}}{\text{Conc-II}_{SCII}}$$

thereby determining the relative CN.

25. *(new)* The method of claim 1 wherein the quantity in the sample in step (4) is the number of copies of NucSeqI or NucSeqII obtained from the respective standard curves in which the quantity or relative dilution of NucSeqI' or NucSeqII', expressed as copy number, is plotted on the X-axis.

26. *(new)* The method of claim 1 wherein the concentration in the sample in step (4) is the molar or weight concentration of NucSeqI or NucSeqII obtained from the respective standard curves in which the concentration or relative dilution of NucSeqI' or NucSeqII' is plotted on the X-axis.

27. *(new)* The method of claim 24, wherein the quantity in the sample in step (4) is the number of copies of NucSeqI or NucSeqII obtained from the respective standard curves in which the quantity or relative dilution of NucSeqI' or NucSeqII', expressed as copy number, is plotted on the X-axis.

28. *(new)* The method of claim 24, wherein the concentration in the sample in step (4) is the molar or weight concentration of NucSeqI or NucSeqII obtained from the respective standard curves in which the concentration or relative dilution of NucSeqI' or NucSeqII' is plotted on the X-axis.